Antitumor Effect of Trastuzumab for Pancreatic Cancer with High HER-2 Expression and Enhancement of Effect by Combined Therapy with Gemcitabine

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Abstract

**Purpose:** The purpose of the present study was to evaluate whether trastuzumab has antitumor effect against pancreatic cancer and whether this effect is concordant with levels of HER-2, which is reportedly overexpressed in pancreatic cancer. We also investigated whether the effect is potentiated in combined therapy with gemcitabine.

**Experimental Design:** Using immunohistochemistry and FACScan, we analyzed HER-2 expression in 16 pancreatic cancer cell lines. The *in vitro* antiproliferative effect of trastuzumab, alone and in combination with gemcitabine, was examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The *in vitro* antibody-dependent cell-mediated cytotoxicity of trastuzumab was investigated by ⁵¹Cr release assay. The *in vivo* antitumor effect of trastuzumab, alone and in combination with gemcitabine, was evaluated in nude mouse xenograft growth. The survival benefit was evaluated in a Capan-1 orthotopic implanted nude mouse model.

**Results:** HER-2 expression of 2+ or more was observed in 10 and of 3+ in 2 of the 16 cell lines. No *in vitro* growth-inhibitory effect of trastuzumab was found in any cell line, but trastuzumab induced antibody-dependent cell-mediated cytotoxicity in proportion to HER-2 expression level. Trastuzumab inhibited tumor growth in Capan-1 (HER-2: 3+) xenografts and prolonged survival in the orthotopic model. These effects were increased by combined therapy with gemcitabine. In contrast, trastuzumab exhibited no antitumor effect against PANC-1 (HER-2: 1+) or SW1990 (HER-2: 2+) xenografts.

**Conclusions:** The antitumor effect of trastuzumab in pancreatic cancer with high HER-2 expression was shown *in vitro* and *in vivo*. Clinical application of trastuzumab is expected in pancreatic cancer with 3+ HER-2 expression.

Today, pancreatic cancer is one of the most lethal cancers in western countries as well as in Japan (1, 2). Despite recent advances in diagnostic modalities and therapeutic strategies, the prognosis is still poor. It has been reported that <4% of patients with adenocarcinoma of the pancreas survive for 5 years after diagnosis (3). This is attributable in large part to difficulties in diagnosis, the aggressiveness of pancreatic cancer, the high incidence of metastasis, and the lack of an effective therapeutic agent. The development of novel therapeutic agents has therefore been a focus of expectation.

The HER-2/neu oncogene encodes a 185-kDa transmembrane glycoprotein receptor, p185HER-2, which has tyrosine kinase activity and partial homology with the epidermal growth factor receptor (4, 5). Overexpression of HER-2/neu at the gene or protein level is reportedly observed in 20% to 30% of breast cancers, and these tumors are associated with poor prognosis and resistance to chemotherapy (6, 7).

Trastuzumab, a recombinant humanized IgG1 monoclonal antibody against HER-2/neu oncoprotein (HER-2), is currently used for the treatment of patients with metastatic breast cancer whose tumor overexpresses HER-2 (8). The mechanisms of the antitumor effect of trastuzumab include direct growth inhibition induced by suppressing signal transduction through HER-2-trastuzumab binding, antibody-dependent cell-mediated cytotoxicity, and antiangiogenic effect (9–13).

In several studies of HER-2 overexpression in pancreatic cancer, its incidence has been reported to vary widely (10-82%; refs. 14–16). These reports also showed that, unlike in breast cancers, these tumors are associated with poor prognosis and resistance to chemotherapy (6, 7).

One phase II clinical trial of trastuzumab for pancreatic cancer has been conducted (17). This trial showed only 6% response (2 of 32 cases) to combined therapy with trastuzumab and gemcitabine in patients with metastatic pancreatic cancer, which is not superior to therapy with gemcitabine alone (18). However, in addition to the patients with 3+ expression of HER-2...
HER-2, the patients enrolled in the trial also included patients with 2+ expression of HER-2, who accounted for ~90% (30 of 34) of the cases studied. The true clinical advantage of trastuzumab in patients with HER-2-overexpressing pancreatic cancer therefore remains unclear.

The purpose of the present study was to evaluate whether trastuzumab has antitumor effect against pancreatic cancer and how this effect is concordant with HER-2 expression. We also investigated whether this effect is potentiated in combined therapy with gemcitabine.

Materials and Methods

Pancreatic cancer cell lines and culture conditions. Sixteen human pancreatic cancer cell lines were used for the present series of studies. SW1990 and RWP-1 were kindly given by Dr. Young-Sik Kim (University of San Francisco, Veterans Administration Medical Center, San Francisco, CA). PCI series (PCI-6, PCI-10, PCI-19, PCI-24, PCI-35, PCI-43c, PCI-55, PCI-64, and PCI-79) were kindly provided by Dr. T. Yoshiki (Hokkaido University, Hokkaido, Japan). Capan-1, Capan-2, MiaPaca-2, and PANC-1 were purchased from American Type Culture Collection (Manassas, VA) and OCUP-AT was established in our laboratory. All cell lines were maintained in high-glucose DMEM (Bioproducts, Inc., Walkersville, MD) supplemented with 10% fetal bovine serum (Life Technologies, Inc., Grand Island, NY) and 0.5 mmol/L sodium pyruvate (Bioproducts). All cells were cultured at 37°C in 5% CO2 in incubators with 100% humidity.

Chemicals. Trastuzumab (Herceptin, Genentech, Inc., South San Francisco, CA) was obtained from Chugai, Inc. (Tokyo, Japan) and gemcitabine was kindly provided by Eli Lilly Japan (Kobe, Japan). We used a recombinant humanized antibody against influenza virus (IgG1) as a control IgG, which was kindly provided by Dr. Y. Suzuki (Hokkaido University).

Flow cytometric analysis. The expression of HER-2 on pancreatic cancer cell lines was examined by indirect immunofluorescence using a FACScan (CellQuest FACSCalibur, Becton Dickinson, Inc., Mountain View, CA).

Fig. 1. Expression of HER-2 in four representative pancreatic cancer cell lines as assessed by immunohistochemistry and FACScan analysis. A. 3+ staining in Capan-1, 2+ staining in SW1990, 1+ staining in PANC-1, and no staining in PCI-6. Original magnification, ×400. B, cell surface expression of HER-2 by FACScan analysis. Capan-1 had high levels of HER-2 expression (MFI > 80) and PCI-6 had low levels of HER-2 expression (MFI < 10). The other cell lines had moderate levels (10 < MFI < 50).
View, CA). Cells (1 x 10^6/mL) were incubated in PBS with trastuzumab or IgG for 2 hours at 37°C. The bound immunoglobulin was detected by mouse anti-human immunoglobulin conjugate to FITC. Fluorescence was measured by flow cytometry using a FACSscan analyzer. Using the software, the percentage of positive cells and mean fluorescence intensity (MFI) were calculated and compared with isotype-matched control-stained cells.

**Immunohistochemistry.** For immunohistochemistry, cells were centrifuged into a cell pellet and embedded in paraffin. Immunoperoxidase staining of thin sections for HER-2 was done using a commercially available HercepTest (DAKO, Inc., Copenhagen, Denmark) in accordance with the manufacturer's instructions. HER-2 staining was quantified as follows: 0, no membrane staining; 1+, barely perceptible staining not totally encircling the cell membrane; 2+, light to moderate staining totally encircling the cell membrane; 3+, moderate to strong staining totally encircling the cell membrane. Cell lines with perceptible staining not totally encircling the cell membrane; 2+, light staining; 3+, moderate staining. In each cell line, 1 to 3+ staining showed in at least 10% of cells was classified as overexpressing HER-2.

**In vitro proliferation assay.** Cell growth was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (19). Briefly, 5,000 viable cells were seeded into flat-bottomed 96-well plates in triplicate and allowed to adhere overnight. They were then treated with the intended doses of trastuzumab (0-100 μg/mL) with or without gemcitabine (0-1,000 ng/mL). After 3 days of incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added to a final concentration of 0.5 mg/mL and the cells were incubated for a further 2 hours under the same conditions. The culture plate was then centrifuged at 200 x g for 5 minutes and the supernatant was removed, after which DMSO was added for reaction and the absorbency was measured with a microplate reader (model 550, Bio-Rad Laboratories, Hercules, CA) and calculated using the supplied software.

**Preparation of effector cells.** The peripheral blood mononuclear cells used as human effector cells were isolated from heparinized whole blood obtained from healthy volunteers by Ficoll-Hypaque density gradients (20). After three washes in PBS, the cells were resuspended in RPMI 1640 (Sigma-Aldrich, Inc., St. Louis, MO) including 10% heat-inactivated fetal bovine serum, and used for in vitro assay for antibody-dependent cell-mediated cytotoxicity.

**In vitro antibody-dependent cell-mediated cytotoxicity.** Assay for antibody-dependent cell-mediated cytotoxicity was done using the 51Cr release method as reported previously (21). Briefly, target cells (1 x 10^6) were labeled with 3.7 MBq of Na$_2$CrO$_4$ (Amersham Biosciences, Inc., Tokyo, Japan) at 37°C for 1 hour. Aliquots of 51Cr-labeled target cells were dispensed into 96-well round-bottomed plates (1 x 10^3 cells) in quadruplicate and incubated with serial dilutions of antibodies (100 μL) for 20 minutes at room temperature. Increasing amounts of effector cells were added to reach E:T ratios ranging from 5 to 50 in a final volume of 200 μL. The plates were then placed in incubators for 8 hours at 37°C in 5% CO$_2$, after which 100 μL supernatant was collected from each well and radioactivity was counted in a γ-counter. All procedures were done without adding interleukin-2. The percentage cytotoxicity was calculated using the following formula: specific lysis (%) = (E - S) / (M - S) x 100, where E is experimental release (counts/min in the supernatant from target cells incubated with antibody and effector cells), S is spontaneous release (counts/min in the supernatant from target cells incubated with medium alone), and M is maximum release (counts/min released from target cells lysed with 1 mol/L HCl).

**In vivo antitumor effect of trastuzumab alone in xenografted nude mouse model.** Female BALB/c nude mice (4 weeks old) were used. The ethical standards of the experiment were approved by the Animal Research Committee of Osaka City University Graduate School of Medicine, and the animals were maintained in accordance with institutional guidelines. The mice were acclimatized at the Animal Facility of Osaka City University Graduate School of Medicine for 1 week.

To produce tumors, pancreatic cancer cells were harvested from subconfluent cultures by treatment with 0.25% trypsin and 0.02% EDTA. Trypsinization was stopped with medium containing 10% fetal bovine serum and the cells were washed once in serum-free medium and resuspended in DMEM. Only single-cell suspensions with >90% viability were used for injection.

Capan-1 was selected as a cell line with high HER-2 expression, SW1990 as one with moderate expression and PANC-1 as one with weak expression. In each cell line, 1 x 10^7 cells in 200 μL DMEM were inoculated s.c. into the left flank of BALB/c nude mice. Treatment was initiated on the 7th to 10th days after cell injection, when the xenografts had reached a mean size of 0.1 to 0.15 cm$^3$

The mice were treated for 4 weeks with twice weekly i.p. injection of trastuzumab (0.1, 1, 10, and 30 mg/kg) and control IgG (10 mg/kg). Treated mice were closely monitored for any signs of progressive disease and sacrificed if they became moribund. Tumors were measured every 3 to 4 days and tumor volume was calculated using the formula: largest diameter x (smallest diameter)$^2$ x 0.5.

**In vivo antitumor effect of trastuzumab in combination with gemcitabine.** We also investigated the effect of trastuzumab in combination with gemcitabine against Capan-1 xenografts. The sufficient dosage of trastuzumab was determined by initial in vivo experiment. Capan-1 xenografted BALB/c nude mice were randomly separated into four groups: (a) twice weekly i.p. injection of trastuzumab (10 mg/kg) alone, (b) twice weekly i.p. injection of gemcitabine (100 mg/kg) alone, (c) twice weekly i.p. injection of both trastuzumab (10 mg/kg) and gemcitabine (100 mg/kg), and (d) twice weekly i.p. injection of control IgG (10 mg/kg) and PBS as control. All groups were treated for 3 weeks. Tumor volume was calculated as in the initial experiment.

Subsequently, to confirm the survival benefit of trastuzumab and gemcitabine, a Capan-1 orthotopic implantation model was used as described previously (22). When tumor growth was recognized by palpation on the 10th day after implantation, mice with Capan-1 tumor orthotopically implanted in the pancreas were divided into four groups, treated as in the protocol outlined above, and their survival periods were recorded.

**Statistical analysis.** Group data are presented as mean ± SD. Differences between the two groups in in vitro antibody-dependent cell-mediated cytotoxicity assays were analyzed by unpaired (or, when appropriate, paired) Student’s t test. The potential of trastuzumab and gemcitabine for inhibition of in vivo tumor growth was analyzed using Tukey’s HSD test. The level of significance was set at the P < 0.05. In the

<p>| Table 1. Result of immunohistochemistry and FACSscan analysis of HER-2 expression in pancreatic cancer cells |
|-----------------|-----------------|-----------------|</p>
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*Assayed by HercepTest.*
survival model, the Kaplan-Meier method was used to analyze survival times and log-rank tests were done for purposes of comparison. Statistical analysis was carried out using SPSS software (SPSS, Inc., Chicago, IL).

Results

**HER-2 expression in pancreatic cancer cell lines.** Cell surface HER-2 expression was assessed by immunohistochemistry using the HercepTest in 16 pancreatic cancer cell lines. Typical immunohistochemistry stains are shown in Fig. 1A. Capan-1 cells showed 3+ staining, whereas SW1990 and PANC-1 showed 2+ and 1+ staining, respectively. Only PCI-6 cells showed no staining. The results of immunohistochemistry are summarized in Table 1.

Cell surface expression of HER-2 was also assessed by FACScan analysis (Fig. 1B; Table 1). The MFIs of Capan-1, SW1990, PANC-1, and PCI-6 were 83.8, 32.4, 12.9, and 9.8, respectively. Of the 16 pancreatic cancer cell lines, 2 had high levels of HER-2 expression (MFI > 80), 13 had moderate levels (MFI between 10 and 50), and only 1 had a low level (MFI < 10).

The results of FACScan and immunohistochemistry are summarized in Table 1. Comparison between the results of immunohistochemistry and FACScan shows that both Capan-1 and PCI-79, which had scores of 3+ in immunohistochemistry, had MFIs of >70. The corresponding values for cell lines with immunohistochemistry scores of 1+ and 2+ were between 10 and 50. PCI-6, which had a MFI of <10, did not stain in the HercepTest.

**In vitro effect of trastuzumab and gemcitabine on pancreatic cancer cell growth.** From the results of FACScan and immunohistochemistry, we selected Capan-1 as a cell line with a high level of HER-2 expression (3+), SW1990 as one with a moderate level (2+), PANC-1 as one with a weak level (1+), and PCI-6 as one with a low level (0).

Trastuzumab alone did not affect the growth of any pancreatic cancer cell line at any concentration (0-100 µg/mL) and no difference in growth inhibition was observed in relation with different HER-2 expression levels (Fig. 2).

Single treatment with gemcitabine produced in vitro antiproliferative effect against all pancreatic cancer cell lines in a dose-dependent manner. No additional or synergic effect was achieved in any cell line by adding trastuzumab to the medium containing gemcitabine (Fig. 2).

**In vitro antibody-dependent cell-mediated cytotoxicity activity induced by trastuzumab.** Trastuzumab with effector cells induced antibody-dependent cell-mediated cytotoxicity activity in Capan-1, SW1990, and PANC-1. Dose-dependent pancreatic cancer cell cytotoxicity was observed in Capan-1 (HER-2: 3+), SW1990 (HER-2: 2+), and PANC-1 (HER-2: 1+), but no such

Fig. 2. *In vitro* growth-inhibitory effect of trastuzumab and gemcitabine against four pancreatic cancer cell lines. Cell growth was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Capan-1, SW1990, PANC-1, and PCI-6 were used. These cells were treated with the intended doses of trastuzumab (0-100 µg/mL) with or without gemcitabine (0-1,000 ng/mL). After 3 days of incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyldihexafluoroborate assay was done. Trastuzumab showed no growth-inhibitory effect against any pancreatic cancer cell lines regardless of HER-2 expression level. Gemcitabine inhibited the growth of all pancreatic cancer cell lines in a dose-dependent manner. When pancreatic cancer cells were cultured with both trastuzumab and gemcitabine, growth inhibition was seen to the same degree as with gemcitabine alone. Points, mean; bars, SD.
in vitro activity was observed in PCI-6 (HER-2: 0). Although no antiproliferative effect was observed in trastuzumab, antibody-dependent cell-mediated cytotoxicity activity proportional to HER-2 expression was found in pancreatic cancer cells (Fig. 3). This activity was stronger in cell lines with 3+ HER-2 expression than in those with scores of 1+ or 2+. Specifically, under conditions of trastuzumab concentration of 0.1 μg/mL and E:T ratio of 50, antibody-dependent cell-mediated cytotoxicity activity in Capan-1 (61.6 ± 4.0) was significantly higher than in SW1990 (32.5 ± 1.5) or Panc-1 (32.1 ± 1.4; P < 0.001).

In vivo antitumor effect of trastuzumab alone in xenografted nude mice. The growth of Capan-1 xenografts (n = 8 per group) was significantly inhibited by i.p. injection of 1, 10, and 30 mg/kg trastuzumab compared with control IgG or 0.1 mg/kg trastuzumab after day 21 (Fig. 4A). In detail, at 35 days, the mean volumes were 545.1 ± 82.5, 507.2 ± 76.8, and 500.0 ± 31.5 mm³, respectively, in groups receiving trastuzumab 1, 10, and 30 mg/kg, which were significantly smaller than that in control group (1,040.4 ± 168.1 mm³; P < 0.001). On the other hand, the mean volume in trastuzumab 0.1 mg/kg group was 1,045.9 ± 186.7 mm³, which is not significantly different compared with that in control (P = 0.97).

In SW1990 (HER-2: 2+; n = 6 per group) and PANC-1 (HER-2: 1+; n = 8 per group), however, trastuzumab produced no inhibitory effect against tumor growth at any dosage, including at 10 mg/kg (Fig. 4B and C).

No toxic effects, such as body weight loss, vomiting, or dermatitis, were observed in any of the trastuzumab-treated groups.

Antitumor effect of trastuzumab in combination with gemcitabine in Capan-1 xenografted nude mice. Tumor growth in nude mice treated with either trastuzumab alone (n = 10) or gemcitabine alone (n = 10) was significantly inhibited compared with control (n = 10). At 28 days, the mean tumor volume was 411 ± 52.2 mm³ in the trastuzumab group and 253 ± 74.2 mm³ in the gemcitabine group, both significantly smaller than in the control group (961 ± 170 mm³; P < 0.001). Furthermore, combined treatment with trastuzumab and gemcitabine (n = 10) produced reductions in tumor volume (69.8 ± 16.3 mm³ at 28 days), a significant antitumor effect compared with single treatment with either drug or with control (P < 0.001; Fig. 5).

Survival benefit of trastuzumab in combination with gemcitabine in Capan-1 orthotopically implanted nude mice. The median survival times of Capan-1 orthotopically implanted nude mice were 72, 82, 86, and 104 days, respectively, in group receiving control, trastuzumab alone (10 mg/kg), gemcitabine alone (100 mg/kg), and combined treatment with trastuzumab and gemcitabine treatment (n = 10 per group; Fig. 6). Median survival time in the trastuzumab group and the gemcitabine group was significantly longer than in the control group (control versus trastuzumab, P = 0.0016; control versus gemcitabine, P < 0.001). Combined treatment significantly prolonged the survival time compared with the two single treatment groups and the control group (combination versus trastuzumab, P = 0.0018; combination versus gemcitabine, P = 0.0043; combination versus control, P < 0.001).

Discussion

The present study made several novel findings relevant to the clinical therapeutic application of trastuzumab against
pancreatic cancer. Firstly, it was found that HER-2-overexpressing pancreatic cancer cells can be killed by antibody-dependent cell-mediated cytotoxicity induced by trastuzumab rather than by direct inhibitory effect against cell growth and that this activity correlates with cell surface HER-2 expression levels. *In vivo* assay showed that trastuzumab had significant antitumor effect against Capan-1 xenograft, which showed high levels of HER-2 expression. Secondly, *in vivo* assay showed that trastuzumab produced an additive effect in combination with gemcitabine. This combined therapy not only showed growth-inhibitory effect in Capan-1 xenografts but also prolonged survival time in Capan-1 orthotopically implanted nude mice.

In the clinical practice of breast cancer, trastuzumab is effective in tumors that have high HER-2 expression reflected in immunohistochemistry scores of 3+ and that are susceptible to gene amplification by fluorescence *in situ* hybridization. The antitumor effect of trastuzumab in breast cancer is reported to involve two main mechanisms: direct inhibition against cell growth and antibody-dependent cell-mediated cytotoxicity (9–11). In the present study, regardless of the degree of HER-2 expression, trastuzumab showed no direct cell proliferative inhibition against pancreatic cancer cell lines. Direct inhibitory effect of trastuzumab has been reported in various malignant tumors, such as lung, pancreas, and colon cancer and glioblastoma (23–26). Other reports have found, in contrast, that trastuzumab has no direct inhibitory effect in gastric cancer (27, 28). These findings suggest that, regardless of HER-2 overexpression, the HER-2 signaling pathway may not play a major role in regulating cell growth and that trastuzumab may not induce direct cell growth inhibition against certain tumors.

We therefore used pancreatic cancer cell lines to investigate whether trastuzumab can induce antibody-dependent cell-mediated cytotoxicity, which is the other mechanism of the antitumor effect of trastuzumab in breast cancer. It was found that trastuzumab induced this *in vitro* activity against HER-2-positive pancreatic cancer cells, which are those with HER-2 scores of 1+ or more in immunohistochemistry. The highest activity was against Capan-1 and seen even at low trastuzumab concentrations of 0.01 μg/mL. Activity against pancreatic cancer cells with 1+ or 2+ HER-2 was less than against Capan-1 cells. Meanwhile, *in vivo* assays showed that trastuzumab displayed antitumor effect at a dose of 0.1 mg/kg against Capan-1 xenografts, but no effect against SW1990 (HER-2: 2+) or PANC-1 (HER-2: 1+) xenografts even at 10 mg/kg. These results indicate that a high level of HER-2 expression of the order of 3+ in immunohistochemistry is needed to obtain antitumor effect against pancreatic cancer with trastuzumab.

A previous *in vivo* study found that trastuzumab completely suppresses the tumor growth of HER-2-overexpressing breast cancer in xenograft-bearing nude mice (29). Although the present study used the same dosage of trastuzumab as the previous study, the growth inhibition remained at only 50% of control. Why was antitumor activity in pancreatic cancer less than in breast cancer? Clynes et al. showed that the antitumor activity of trastuzumab against breast cancer xenograft is

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**Fig. 4.** Antitumor activity of trastuzumab against pancreatic cancer xenografts. BALB/c nude mice bearing xenograft were treated for 4 weeks with twice-weekly i.p. injection of trastuzumab and control IgG. A, Capan-1 xenografts (*n* = 8 per group): trastuzumab was given at doses of 0.1, 1, 10, and 30 mg/kg; the control group was treated with anti-influenza virus antibody (IgG) at 10 mg/kg. B, SW1990 xenografts (*n* = 6 per group): trastuzumab was given at doses of 1 and 10 mg/kg; the control group was treated with IgG at 10 mg/kg. C, PANC-1 xenografts (*n* = 8 per group): trastuzumab was given at doses of 1 and 10 mg/kg; the control group was treated with IgG at 10 mg/kg. Tumors were measured every 3 to 4 days and tumor volume was calculated using the formula: largest diameter × (smallest diameter)^2 / 2. Points, mean; bars, SD. ***, *P* < 0.001, statistically significant differences between the control group and the trastuzumab groups (1, 10, and 30 mg/kg) in Capan-1 xenografts at 35 days (Tukey’s HSD test).
reduced to <50% when Fc receptor knockout nude mice are used (30). This result indicates that the antitumor effect of trastuzumab against breast cancer depends half on antibody-dependent cell-mediated cytotoxicity and half on direct cell growth inhibition. It can be the reason why the antitumor effect of trastuzumab in pancreatic cancer was less than in breast cancer that trastuzumab has no antiproliferative effect against pancreatic cancer cells but can induce antibody-dependent cell-mediated cytotoxicity.

We also investigated whether an additive or synergic effect would be seen when trastuzumab was used in combination with gemcitabine, which is in current clinical use as a chemotherapeutic agent for advanced pancreatic cancer. In an in vitro study, gemcitabine did show cell growth inhibition against pancreatic cancer cells at a concentration of 10 to 50 ng/mL, but no additive effect was observed in combination with trastuzumab. In an in vivo study using Capan-1 xenografts, on the other hand, it was found that combined therapy produced significantly stronger growth-inhibitory effect than single therapy with either drug or with control (P < 0.001). Points, mean; bars, SD. * P < 0.001, statistically significant differences between groups (Tukey’s HSD test).

cancer. Although most chemotherapeutic agents damage the immune function, it has been reported that gemcitabine treatment is not immunosuppressive and may enhance response to specific immunotherapy (31, 32). Gemcitabine may therefore be suitable for combination with certain kinds of immunotherapy, including antibody-dependent cell-mediated cytotoxicity.

A clinical phase II study of combined therapy with gemcitabine and trastuzumab has already been carried out by Safran et al., but the response rate was reported to be only 6% in cases of metastatic pancreatic cancer with 2+ or 3+ HER-2 expression, which did not exceed the response rate of treatment with gemcitabine alone (18). The Safran et al. study did include pancreatic cancer patients whose tumors had HER-2 expression of 2+ or more in the HercepTest, but most of the cases [30 of 34 (88%)] had 2+ and only four cases had 3+. In the present in vivo study, a significant antitumor effect of trastuzumab was observed only in pancreatic cancer xenografts with 3+ HER-2 expression and not in xenograft with expression level of 1+ or 2+. These results may mean that indication of trastuzumab therapy for pancreatic cancer should be limited to cases with HER-2 expression of 3+ in actual clinical application and suggest that adequate therapeutic response may be expected from these 3+ patients.

The present study in 16 pancreatic cancer cell lines showed that if an immunohistochemistry score of 2+ or more for HER-2 expression is taken as indicating overexpression, 10 of 16
(62.5%) cell lines had overexpression. However, 3+ expression was observed in only 2 of 16 (12.5%) cell lines. In previous reports, the positive rates for HER-2 expression in pancreatic cancer varied widely (10-82%; refs. 14-16). When the sample is limited to studies using the Herceptin Test, most reports state that a score of more than 2+ is found in 10% to 20% (15, 16). According to the findings of the present study, trastuzumab may be effective in pancreatic cancer therapy against cases with 3+ HER-2 expression, thus posing the problem of a considerably limited range of indication. It is actually speculated that the number of cases with 3+ HER-2 expression may be <10% in the clinical setting, but given a clinical situation in which no superior therapy is currently available for pancreatic cancer, trastuzumab must be seen as an appropriate candidate pancreatic cancer treatment, particularly for cases with 3+ HER-2 expression.

In conclusion, the effect of trastuzumab on pancreatic cancer with a high level of HER-2 expression was shown in vitro and in vivo. The action mechanism was mainly antibody-dependent cell-mediated cytoxicity rather than direct cell growth inhibition. Trastuzumab produced tumor growth inhibition in Capan-1 xenografts and prolonged survival periods in a Capan-1 orthotopic nude mouse model. These effects were increased by combined therapy with gemcitabine. In the future, clinical application of trastuzumab for pancreatic cancer with 3+ HER-2 expression is expected.

References

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